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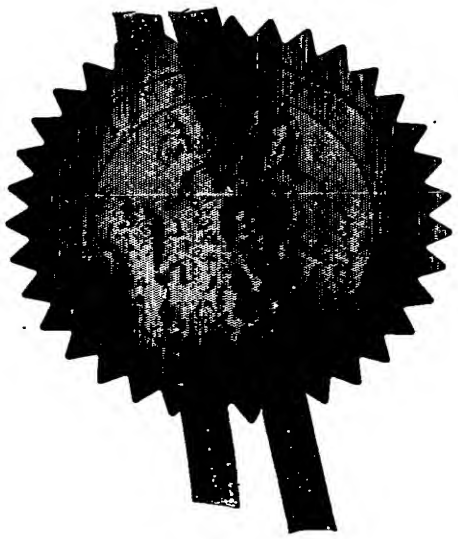

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1. Your reference — P32890-/GTO/BPU

14 NOV 2003

2. Patent application number *(The Patent Office will fill this part in)* — 0326578.2

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

The Queen's University of Belfast
University Road
Belfast BT7 1NN
Northern Ireland

Patents ADP number *(if you know it)* ~~55787860005~~ 00772798001

If the applicant is a corporate body, give the country/state of its incorporation — UK

4. Title of the invention — Cancer Diagnosis and Therapy

5. Name of your agent *(if you have one)* — Murgitroyd & Co

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Scotland House
165-169 Scotland Street
Glasgow G5 8PL
Scotland

Patents ADP number *(if you know it)* 1198015

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Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

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Description	35
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Statement of inventorship and right to grant of a patent (Patents Form 7/77)	
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Request for a substantive examination (Patents Form 10/77)	
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11. I/We request the grant of a patent on the basis of this application.

Signature(s) Date 13 November '03

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

Barry Purdy 0141 307 8400 barry.purdy@murgitroyd.com

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Cancer Diagnosis and Therapy

Technical Field

The invention relates to a novel oncofetal glycoprotein which is expressed in certain tumours, antibodies to the protein, and uses of the antibodies in cancer diagnosis.

Background Art

The cancer phenotype typically displays loss of differentiation, loss of proliferative control and altered cell adhesion molecule expression. Cell surface proteins have been shown to play an important role in cell-cell interactions (eg NCAM), cell-extra-cellular interactions (eg CD44) and cell regulation (eg Notch signaling).

Some of these cell surface proteins have oncofetal expression profiles and as such have been used as

tumour specific diagnostic markers (eg CEA). A further use for antibodies specific for cell surface proteins over expressed in cancer has been in the treatment of cancer by immunotherapy/radioimmunotherapy (eg Herceptin an antibody recognizing HER2).

Statements of Invention

In one aspect, the invention relates to an isolated nucleic acid sequence which comprises a sequence selected from the group consisting of: Sequence ID No.1, Sequence ID No, 2, and Sequence ID No. 3. Typically, the nucleic acid sequence is a DNA sequence. In one embodiment, the nucleic acid sequence consists of a sequence selected from the group consisting of: Sequence ID No. 1, Sequence ID No. 2 and Sequence ID No. 3.

The invention also relates to an isolated protein encoded by the isolated nucleic acid sequences of the invention. Typically, the protein is a cell surface glycoprotein. In one preferred embodiment, the isolated protein is an oncofetal protein expressed by an astrocytoma cell. Typically, the protein has a molecular weight of approximately 200kda. In this specification, the term "protein" should be understood as including amino acid sequences which would more generally be referred to a peptides.

In another aspect, the invention relates to an antibody which binds specifically to the protein of

the invention and any other antibody that competes directly or by stearic hindrance therewith for said protein. Typically, the antibody is a monoclonal antibody. In one embodiment, the antibody is a class M immunoglobulin with a kappa-light chain.

In another aspect, the invention relates to a fragment of the antibody of the invention, which fragment binds specifically to the protein of the invention.

In another aspect, the invention relates to a method of producing an antibody to a protein comprising:

- innoculating an animal with a protein according to the invention, wherein the protein elicits an immune response in the animal to produce the antibody; and

- isolating the antibody from the animal.

In one embodiment, the animal is innoculated with G-CCM cells of ECACC deposit No. 86022702.

In a further aspect, the invention relates to a process for producing a hybridoma, comprising the step of innoculating a suitable subject with a protein according to the invention, or an antigenic fragment thereof, and fusing cells from the subject with a myeloma cell to produce the hybridoma. Typically, the subject is innoculated with G-CCM cells of ECACC deposit No. 86022702.

In a further aspect, the invention relates to a hybridoma cell obtainable according to the above process. In one embodiment, the invention relates to a hybridoma cell of, or derived from, ECACC Deposit No. 03073001.

A deposit of hybridoma cells according to the invention was made at the European Collection of Cell Cultures on 30 July 2003 and accorded the accession number ECACC 03073001.

In another aspect, the invention relates to a monoclonal antibody obtainable from the hybridoma cell of, or derived from, ECACC Deposit No. 03073001.

The invention also relates to a method of detecting an astrocytoma cell in a sample of human cells, which method comprises the step of contacting the cell sample with an antibody of the invention, or a fragment thereof, and detecting those cells which have bound the antibody or fragment, wherein binding of the antibody or the fragment to a cell is indicative of an astrocytoma cell. Typically, the antibody is a monoclonal antibody of the invention.

The invention also relates to a method of detecting a primary breast carcinoma cell in a sample of human cells, which method comprises the step of contacting the cell sample with an antibody of the invention, or a fragment thereof, and detecting those cells

which have bound the antibody or fragment, wherein binding of the antibody or the fragment to a cell is indicative of a primary breast carcinoma cell. Typically, the antibody is a monoclonal antibody of the invention.

The invention also relates to a diagnostic kit for diagnosing the presence of a cell selected from the group consisting of: astrocytoma cells; malignant melanoma secondary tumour cells; and primary breast carcinoma cells, the kit comprising an antibody according to the invention, or a fragment thereof. Typically, the antibody is a monoclonal antibody of the invention. In one embodiment, the antibody of the invention comprises a detectable label. Alternatively, the kit comprises a secondary antibody which specifically binds the antibody of the invention, which secondary antibody comprises a detectable label.

The invention also relates to a biological targeting device comprising an antibody, typically a monoclonal antibody, of the invention, or a fragment thereof, and a therapuetic ligand.

The invention also relates to a therapeutic antibody comprising an antibody, typically a monoclonal antibody, of the invention, or a fragment thereof.

The invention also relates to a method of treating cancer in an individual by inducing apoptosis in cells in the individual which express a protein of

the invention, which method comprises a step of treating an individual with an antibody of the invention, or a fragment thereof. Typically, the antibody is a monoclonal antibody. In one embodiment, the cancer is selected from the group consisting of: malignant astrocytomas ; malignant melanoma secondary tumours; and primary breast carcinomas. Typically, the antibody is humanised.

The invention also relates to a polynucleotide which is anti-sense to an insolated nucleic acid sequence of the invention. In one embodiment, the anti-sense polynucleotide comprises, or consists of, a sequence of Sequence ID No. 4.

The invention also relates to a method of treating cancer in an individual by inducing apoptosis in cells in the individual which express a protein of the invention, which method comprises a step of treating an individual with an anti-sense polynucleotide of the invention. In one embodiment, the cancer is selected from the group consisting of: malignant astrocytomas; malignant melanoma secondary tumours; and primary breast carcinomas. Methods of delivery of anti-sense polynucleotides will be well-known to those skilled in the art of gene therapy.

The monoclonal antibodies of the invention may be the complete antibodies described herein, or fragments thereof. That is, they may be any fragment of a monoclonal antibody of the invention that specifically recognises the protein of the

invention. Such fragments include Fab, F(ab')2, Fab', etc. These fragments ban be prepared by digestion with an enzyme such as papain, pepson, ficin, or the like. The properties of the obtained fragments can be confirmed in the same manner as described herein.

The principle reason for the poor prognosis associated with malignant astrocytomas is recurrence due to invasion of surrounding brain parenchyma by tumour cells with an invasive phenotype. This phenotype displays loss of differentiation, secretion of proteases and altered cell adhesion molecule expression. As part of an investigation into the mechanisms of astrocytoma invasion, monoclonal antibodies (Mab) were raised against cell surface proteins expressed by an anaplastic astrocytoma cell line (G-CCM). One of the antibodies produced (MQ1 Mab) recognizes a previously undescribed cell surface glycoprotein (MQ1). In vitro MQ1 protein expression was found on astrocytomas and fetal astrocytes, with the level of expression increasing with astrocytoma malignancy and decreasing with fetal astrocyte maturity. Immunohistochemistry on histologically normal and neoplastic brain tissue demonstrated that MQ1 protein expression is restricted to astrocytomas (n=52). Other primary brain tumours tested (oligodendrogliomas, neurinomas, PNET, and medulloblastomas) and normal brain cells, including neurons, oligodendrocytes and endothelial cells were MQ1 negative, thus indicating that the MQ1 proteins

have the expression pattern of oncofetal proteins. Similarily a study looking at primary breast carcinomas found 60% were MQ1 positive (n=228). Surrounding normal tissue, fibrocystic disease and fibroadenoma tissue were MQ1 negative. Malignant melanoma secondary tumours to the brain were also found to be strongly MQ1 positive.

A cDNA expression library was synthesized from G-CCM mRNA and screened with the MQ1 antibody. Two positive clones were isolated (Sequence ID No.s 1 and 2) and sequencing data demonstrated that both have a high degree of homology with Jagged1, a human Notch ligand which plays a role in differentiation and determination of cell fate. The library was rescreened with probes generated from the positive clones and further homologous transcripts were isolated including a possible Jagged1 splice variant (Sequence ID No. 3). Northern blotting for a range of cell lines with these probes revealed the presence of two transcripts (approximately 3.5kb & 5.0kb). Subsequent protein studies (immunocytochemistry, immunoblotting and co-immunoprecipitation) indicate that the MQ1 protein has a high degree of homology with, but is not identical to, Jagged1.

This investigation has identified a novel oncofetal glycoprotein with homology to Jagged1. Its tumour specificity together with its potential role in regulating cellular differentiation /apoptosis suggest that it may be a valuable prognostic marker

and therapeutic target.

The invention will be more clearly understood from the following description of some embodiments thereof, given by way of example only, with reference to the following Figures in which:

Fig.1A illustrates confocal microscopy of live G-CCM cells immunolabelled with MQ1 showing recognition of a cell surface epitope;

Fig. 1B illustrates confocal microscopy of permeabilized G-CCM cells immunolabelled with MQ1 showing recognition of an intracellular epitope and localisation of the antigen at areas of cell contact on the cell surface;

Fig 2 shows a comparision of MQ1 expression, by immunocytochemistry and flow cytometry, on a range of fetal astrocyte cultures and astrocytoma cell lines. A-C show immunocytochemistry on live cells of a grade IV, grade III and 16 week gestation fetal astrocytes respectively. D-F chow the corresponding flow analysis with the same cells with the level of MQ1 surface expression estimated as mean channel fluorescence. G shows the results of the flow analysis plotted as a graph. This demonstrates an inverse correlation of cell surface MQ1 protein expression with fetal astrocyte maturity and correlation with astrocytoma grade;

Fig.3 shows immunohistochemistry displaying diffuse

MQ1 positivity throughout A) Grade I astrocytomas B) Grade II astrocytomas C) Grade III astrocytomas & D) focal positivity in grade IV astrocytoma cells palisading an area of necrosis;

Fig.4 shows MQ1 immunocytochemistry showing A) strong MQ1 positivity at the tumour front B) strong MQ1 positivity in reactive astrocytes in adjacent tissue C) GFAP positivity in reactive MS tissue D) MQ1 negative reactive MS tissue;

Fig. 5 shows MQ1 immunohistochemistry of breast carcinoma tissue showing A) strong MQ1 positivity in invasive ductal carcinoma cells surrounded by MQ1 negative stroma B) strong MQ1 positivity in lobular carcinoma surrounded by MQ1 negative stroma;

Fig.6 shows MQ1 immunocytochemistry of G-CCM cells treated with (A) 0.1μm control oligo (B) 0.5μm control oligo .(C) 1.0μm control oligo (D) 0.1 μm anti-sense MQ1 oligo (E)0.5μm anti-sense MQ1 oligo and (F) 1.0 μm antisense oligo, showing that MQ1 anti-sense oligo knocks out MQ1 protein expression at concentrations of 0.5 and 1.0 μm;

Fig. 7 shows an immunoblot indicating Parp cleavage of oligo-treated G-CCM cells;

Fig. 8 shows immunocytochemistry (ICC) detection of cleaved Caspase 3 following oligo treatmentl; and

Fig. 9 shows G-CCM cells labelled with MQ1 antibody

by ICC, 24 hours post-treatment with control and anti-sense oligonucleotides in which:

(A) control oligo 0.1μM
(B) control oligo 0.5μM
(C) control oligo 1.0μM
(D) Anti-sense oligo 0.1μM
(E) Anti-sense oligo 0.5μM
(F) Anti-sense oligo 0.1μM

MATERIALS AND METHODS

Materials

All cell culture reagents were obtained from Gibco BRL (Paisley, UK) with the exception of the hypoxanthine, aminopterin and thymidine (HAT) and the hypoxanthine and thymidine (HT) that were obtained from Sigma (Poole, Dorset, UK). The secondary and negative control antibodies were supplied by Dako (Bucks, UK). The PARP and Caspase3 antibodies were purchased from Sigma (Poole, Dorset, UK) and the Protein-A Sepharose CL4B from Pharmacia Biotech (Herts, UK). PTO linked oligonucleotides were obtained from MWG-Biotech (Germany).

Cell culture

The CB109 cell line was established from a glioblastoma multiforme [6] and was a gift from Dr Claude Chauzy (Centre Henri Becquerel, Rouen, France). The G-CCM cell line was derived from a

human anaplastic astrocytoma and was a gift from Dr Ian Freshney (Department of Clinical Oncology, University of Glasgow, UK). The G-CCM cell line is commercially available from the European Collection of Cell Cultures under Deposit No 86022702. The fetal astrocyte cell cultures were a gift from Ms Kim Martin (Department of Neuropathology, Institute of Psychiatry, London, UK). The C6 cell line, derived from a rat glioma , was obtained from Flow Laboratories (Scotland, UK). The skin fibroblast cell culture was initiated in our laboratory from a surgical specimen obtained from the Neurological Unit (Royal Victoria Hospital, Belfast, UK). The remaining glioma cell lines were initiated in our laboratory from surgical specimens received from the Neurosurgical Unit (Royal Victoria Hospital, Belfast, UK) and were used experimentally after 5-10 passages. Tumour grading follows the World Health Organisation classification. Cell lines were incubated at 37oC/5% CO2 in Dulbecco's modified Eagle's medium (DMEM) containing 2mM glutamine, 10% fetal calf serum (FCS), and phenol red. All cell lines were tested for mycoplasma using Hoechst 33258 fluorescent dye and were found to be negative.

Monoclonal antibody production

Mabs were produced utilizing a standardized protocol designed to promote a rapid predominantly IgG response. In brief, a BALB/c mouse was inoculated intra-peritoneally with 5x106 G-CCM cells in 1ml of Freund's complete adjuvant. Similar doses

emulsified in Freund's incomplete adjuvant were administrated 14 and 28 days later to boost the immune response. Four days after the final booster inoculation the mouse was killed, its spleen aseptically removed and the splenocytes induced to fuse with NSO myeloma cells (at a ratio 5:1) using polyethylene glycol. The resulting fusion products were suspended in a selective, HAT-supplemented, growth medium (RPMI-1640 medium containing 10mM L-glutamine, 1% sodium pyruvate, 100 iu/ml penicillin, 100□g/ml streptomycin and 20% Myoclone FCS) and seeded into 96-well plates. The medium, from the viable hybridomas produced, was screened by indirect immunofluorescence against live and acetone-fixed G-CCM cells. Those showing specific recognition were recloned three times, to ensure monospecificity, in HT-supplemented growth medium and stored in liquid nitrogen. The hybridoma cell line MQ-1, which produced an antibody recognizing a cell surface antigen was propagated as an ascitic tumour in BALB/c mice previously immunosuppressed with Pristane. The ascitic fluids were collected, centrifuged and frozen at -20oC until use.

The positively labelling Mabs were isotyped for their class and light chains using a monoclonal antibody isotyping kit.

Immunofluorescence

Hybridoma medium (neat) or ascites fluid (diluted 1:200 in PBS) was incubated with living cells, grown

to 90% confluence on coverslips, for 40 min at room temperature (RT). After washing, the cells were fixed in acetone at -20oC for 10 min followed by rehydration in PBS and incubation with an FITC-conjugated rabbit antimouse antibody (FITC-RAM) for 30 min at RT. After two further washes the cells were mounted on a glass slide, in a drop of Citifluor, and examined using a Zeiss immunofluorescence microscope or a Biorad confocal microscope. Incubations in PBS without primary antibody were used as negative controls. The fluorescent labelling of positive cells was subjectively rated from low intensity (+) to high intensity (++++).

Flow Cytometry

A preliminary study (results not shown) comparing the expression of MQ-1 protein on cells removed enzymatically (trypsin) and non-enzymatically (0.53 mM EDTA in PBS) from culture flasks, revealed that the MQ1 protein epitope was trypsin-resistant.

Cultured cells were removed from the flasks by trypsinization, counted and aliquoted into centrifuge tubes at a concentration of 5x105 cells per tube. Triplicate samples were incubated in excess ascitic fluid in 200□l of serum free medium containing 1% bovine serum albumin (SFM/BSA) for 40 min at RT with gentle agitation. Following 2 washes in SFM the cells were incubated in an FITC-RAM antibody for 30 min at RT with gentle

agitation. The cells were then washed twice in SFM and fixed in PBS containing 1% para-formaldehyde. The samples were analysed within 48 hr of fixation, using a Coulter EPICS Elite flow cytometer. Negative controls were incubated with an antibody raised against Aspergillus niger glucose oxidase, an enzyme not present or inducible in mammalian cells. The consistency of the mean channel fluorescence measurements between sample batches was checked using EPICS Immuno-Brite standards.

Immunohistochemistry

On receipt the tissue was fixed in 10% formalin prior to routine embedding in paraffin wax using a Tissue Tex VIP (Miles Scientific) automated processor. The paraffin blocks were sectioned at a thickness of 6mm and mounted onto 3-aminopropyltriethoxysilane-coated slides. The tissue sections for indirect immunohistochemistry were processed using an avidin-biotin peroxidase complex (ABC) method. The tissue was dewaxed in xylene and rehydrated before endogenous peroxidase activity was blocked by a 10min incubation in 3% H2O2 in methanol at room temperature (RT). To counter antigen masking, due to the formalin fixation, the tissue was pretreated with microwave irradiation to promote antigen retrieval. The sections were washed in distilled water and placed in 0.01M Tri-Na citrate pH7.8 and irradiated in a Miele microwave oven for 6min (2x3min) at 450W (the optimal toime and intensity of irradiation was

determined from preliminary studies). After incubation in PBS containing 5% normal rabbit serum for 10min at RT the sections were incubated in MQ1 ascites (diluted 1:50 in PBS) at 4C overnight. Following 2x5min washes in PBS the sections were incubated in biotinylated rabbit anti-mouse IgM diluted 1:400 in PBS for 40min at RT. After further washes in PBS, a streptavidin-biotin complex linked to peroxidase was added to the sections and incubated for 40min at RT. The peroxidase reaction was developed in 0.1% diaminobenzidine in PBS activated with 1% H2O2. After washing in water, the sections were counterstained in haematoxylin, dehydrated through graded alcohols, cleared in xylene and mounted in DPX. In addition to negative controls, incubated with a primary antibody raised against Aspergillus niger glucose oxidase, positive controls of histologically normal brain and astrocytoma tissue were included with every batch.

cDNA Expression Library and screening.

G-CCM Cell cDNA Library Synthesis

A Total RNA isolation from G_CCM cells

This was performed using Tel-Test RNA Stat-60, following their guidelines. Web Site www.isotexdiagnostics.com/rna_stat-60_reagent.html

B mRNA Purification from Total RNA

This was performed using Invitrogen's FastTrack 2.0 Kit, following their guidelines. Web Site

www.invitrogen.com/content.cfm?pageid=3443&cfid=330835&cftoken=53475959#FastTrack

C cDNA Library Synthesis from mRNA

This was performed using a Stratagene cDNA synthesis kit (following their protocol).

Stratagene ZAP Express cDNA Synthesis Kit Instruction Manual

www.stratagene.com/manuals/200403.pdf

RESULTS

Antibody Production

The fusion resulted in the production of five viable antibody secreting hybridomas which screened positively by immunofluorescence microscopy on acetone fixed G-CCM cells. Of these, one (hybridoma MQ1) was found to secrete an antibody which was isotyped as a class M immunoglobulin with a kappa-light chain. This antibody recognizes a cell surface epitope, showing punctate labelling, on live G-CCM cells. Further examination by confocal microscopy confirmed the cell surface labelling of live G-CCM cells and revealed the presence of an intra-cellular epitope in permeabilized cells (Figure 1A&B). In addition examination of the permeabilized cells demonstrated localisation of labelling at focal adhesion points on the cell surface.

Immunocytochemistry

A range of cell lines was examined by indirect immunofluorescence for the presence of the MQ-1 antigen (Table 1).

Table 1

CELL LINE	TISSUE SOURCE	MQ1 LABELLING
Fibroblasts	Normal skin	–
C6	Rat glioma	–
FA 10 weeks	Human fetal astrocytes	+
FA 12 weeks	Human fetal astrocytes	+
FA 14 weeks	Human fetal astocytes	+
FA 15 weeks	Human fetal astrocytes	+
FA 16 weeks	Human fetal astrocytes	+
FA 19 weeks	Human fetal astrocytes	+
NP 527/94	Pilocytic astrocytoma (I)	++
NP 396/94	Pilocytic astrocytoma (I)	++
NP 424/94	Astrocytoma (II)	++
NP 676/92	Astrocytoma (II)	++
NP 445/92	Astrocytoma (II)	++
NP 204/92	Astrocytoma (II)	++
NP 482/96	Astrocytoma (II)	++
NP 473/92	Anaplastic astrocytoma (III)	+++
G-CCM	Anaplastic astrocytoma (III)	++++
NP 493/94	Anaplastic astrocytoma (III)	+++
NP 785/96	Anaplastic astrocytoma (III)	+++
NP 402/93	Glioblastoma multiforme (IV)	++++
NP 293/96	Glioblastoma multiforme (IV)	+++
NP 602/91	Glioblastoma multiforme (IV)	++++
NP 536/94	Glioblastoma multiforme (IV)	+++

1	NP 306/92	Glioblastoma multiforme (IV)	++++
2	NP 479/95	Glioblastoma multiforme (IV)	+ + +
3	NP 770/96	Glioblastoma multiforme (IV)	+ + +
4	NP 876/96	Glioblastoma multiforme (IV)	+ + + +
5	NP 39/96	Glioblastoma multiforme (IV)	+ + +
6	CB 109	Glioblastoma multiforme (IV)	—
7	NP 670/92	Glioblastoma multiforme (IV)	—

Table 1. Indirect immunofluorescence on a range of live cell lines and cell cultures with MQ1 antibody.

The results show that the human skin fibroblasts and the C6, rat glioma, cell lines do not express the antigen. The fetal astrocytes and glioma cell lines were positive with the exception of two cell lines (CB109 and NP670/92) derived from glioblastomas multiforme. Under subjective microscopic analysis there appeared to be a variation in labelling intensity between the positive cell lines. The high grade gliomas had a higher labelling intensity than low grade gliomas and fetal astrocytes. This was confirmed by flow cytometry (Figure 2). The results show a progressive increase in MQ-1 antigen expression, as estimated by the mean channel fluorescence, from low to high grade astrocytomas, the expression on grade IV astrocytomas being more than double that of grade I astrocytomas. The fetal astrocytes showed a lower expression than the astrocytoma cell lines, that halved from fetal astrocytes of 12 weeks gestation to 16 weeks gestation.

Immunohistochemistry

The results of the immunohistochemical study on primary brain tumours are summarized in Table 2.

Table 2

Tumour	# Biopsies	MQ1 positivity
Astrocytomas	30	29/30
Neurinoma	3	0/3
Oligodendroglioma	3	0/3
Medulloblastoma	3	0/3
PNET	3	0/3

Table 2 Immunohistochemical analysis of MQ1 immuno-labelling of a range of Primary Brain Tumours showing that of the tumour tissue tested only astrocytomas displayed MQ1 positivity.

The results show that of all the primary brain tumours tested (oligodenrogliomas, PNET etc) only astrocytomas were MQ1 positive.
All pilocytic (grade I) astrocytomas showed a similar staining pattern. There was strong cellular immunostaing of MQ1 proteins which extended to the cellular processes of bipolar cells (Fig3A). The immunopositive cells stood out prominently against a loosely arranged less cellular stroma.
The astrocytomas (grade II) and anaplastic (grade III) astrocytomas revealed a diffuse immunopositivity and the staining pattern was similar in all (Fig 3B&C). There was variation in

the staining pattern of glioblastomas. Out of 16 glioblastomas tested, 1 was unreactive revealing no MQ1 protein expression whereas 14 showed focal positivity and one diffuse immunostaining (Fig 3D). Focal positivity was observed as clusters or groups of positive cells surrounded by unreactive aresas. Tumour cells palisading around areas of necrosis, a characteristic feature of glioblastomas also reveled focal positivity. However tumour giant cells, bizarre cells and clusters of proliferating endothelial cells were negative for MQ1 protein expression. The oligodendroglial cells were negative. Within adjacent grey matter the neurones did not show immunolabelling for the MQ1 proteins. The endothelial cells lining small and large blood vessels in and around tumours of all grades showed no MQ1 protein expression. There was no immunolabelling of lymphocytes in the perivascular spaces. The infiltrating edge of the tumours and the adjacent glial areas showed prominent labelling of large reactive astrocytes (Fig4 A&B)). Such cells revealed multiple processes. However this MQ1 positivity in reactive astrocytes was only found surrounding MQ1 positive tumours, other reactive tissue such as MS tissue that shows prominent reactive astrocytes when labeled for GFAP (FIG 4C) displayed no MQ1 positivity in the 10 biopsies tested (Fig 4D).

In non-CNS tissue tested malignant melanoma and breast 2o to the brain were found to express the MQ1 proteins (Table 3).

Table 3

Tissue	# Biopsies	MQ1 Positivity
Breast 20 (brain)	3	3/3
Breast 1o	228	137/228
Fibroadenoma	5	0/5
Fibrocystic Diease	5	0/5
M.Melanoma2o (brain)	4	4/4

Table 3 Immunohistochemical MQ1 immunolabelling of a range of non-CNS tumours, showing MQ1 positivity in 60% of primary breast tumours and no positivity in fibrocystic diease and fibroadenomas that are non-malignant breast conditions.

Of the primary breast tumours tested 137/228 were MQ1 positive while fibrocystic diease and fibroadenoma tissues, both premalignant conditions displayed no MQ1 positivity. Figure5 shows strong MQ1 positivity in invasive ductal carcinoma cells and lobular carcinoma cells surrounded by MQ1 negative stroma.

Isolation of MQ-1 Clones

Screening of a cDNA expression library (from G-CCM mRNA) with the MQ1 antibody identified two clones with significant homology to the Jagged 1 protein (Sequence ID No's 1 and 2).

Antisense Treatment Protocol

Antisense Oligonucleotide
5'-tgg gga acg cat cgc tgc-3' (Sequence ID No. 4)

Antisense Control Oligonucleotide
5'-tgg gga ccg cat cgc tgc-3' (Sequence ID No. 5)

The PTO linked antisense oligonucleotide was designed against the transcription initiation site and kozac sequence at the beginning of the Jagged1 gene (Accession number AF028593). The control oilgonucleotide was the same 18 mer with one base changed (therefore being the tightest control possible to generate). Both oligonucleotides were synthesized by MWG Biotech. For colony count assays G-CCM cells were seeded out into 24well plates at 50,000 cells/well. The cells were incubated for 24hrs in growth medium and then washed with serum free medium (SFM). The cells were then either treated with lipofectin (Invitrogen Life Technologies) alone following the standard protocol (at 5μl/ml) or lipofectin with the antisense and antisense control oligonucleotides at a range of concentrations (0.1, 0.5 and 1.0 μM) for 16hrs. Following treatment the cells were washed twice with SFM and then incubated in growth medium for 24 and 48hrs. The results (Figure 6) show that treatment with the antisense oligonucleotide at concentrations of 0.5 and 1.0 μM reduced the tumour cell population when compared to the control oligonucleotide and lipofectin alone treatment. To assess whether this was due to the induction of apoptosis similarly treated cells were harvested for their protein and

examined for Parp cleavage (an indicator of apoptosis) by immunoblotting. The results (Figure 7) clearly show a reduction in the level of Parp at 0.5 and 1.0μM antisense oligonucleotide treatment when compared to control oligonucleotide and lipofectin alone treatment. Thus indicating that the antisense oligonucleotide treatment induces apoptosis in the G-CCM cells. To confirm this, treated G-CCM cells were also examined for the presence of cleaved Caspase 3 (another indicator of apoptosis) by immunocytochemistry. The results (Figure 8) show that G-CCM cells treated with 1.0μM displayed caspase 3 cleavage thus indicating that apoptosis was being induced. To demonstrate that these effects were due to the knocking out of the MQ1 proteins by the antisense oligonucleotides, treated cells were examined for the presence of the MQ1 proteins by immunocytochemistry with the MQ1 antibody. The results (Figure 9) show that the expression levels of the MQ1 proteins is reduced by antisense oligonucleotide treatment when compared to the control oligonucleotide.

The discovery highlighted by this work has potential uses as a:

Diagnostic Tool- The antibody clearly distinguishes astrocytomas from other primary brain tumours, normal cells and reactive gliosis. In addition it recognizes 60% of primary breast tumours tested.

Targeting Device- The specificity of the antibody

means it could be used as a targeting devise such as in radioimmunotherapy.

Therapeutic Target- The antibody itself could be used as a therapeutic agent by blocking out signaling through the MQ1/Notch pathway thus inducing apoptosis in astrocytoma cells.

The invention is not limited to the embodiments hereinbefore described which may be varied without departing from the spirit of the invention.

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CLAIMS

1. An isolated nucleic acid sequence which comprises a sequence selected from the group consisting of: Sequence ID No.1, Sequence ID No.2, and sequence ID No 3.

2. An isolated nucleic acid sequence according to Claim 1 in which the nucleic acid sequence is a DNA sequence.

3. An isolated nucleic acid sequence according to Claim 1 or 2 in which the nucleic acid sequence consists of a sequence selected from the group consisting of: Sequence ID No.1, Sequence ID No.2, and Sequence ID No.3.

4. An isolated protein encoded by a nucleic acid sequences according to any of Claims 1 to 3.

5. An isolated protein according to Claim 4 in which the protein is a cell surface glycoprotein.

6. An isolated protein as claimed in Claim 4 or 5 which is an oncofetal protein expressed by an astrocytoma cell.

7. An isolated protein as claimed in any of

Claims 4 to 6 having a molecular weight of approximately 200kda.

8. An antibody which binds specifically to the protein of any of claims 4 to 7, and any other antibody that competes directly or by stearic hindrance therewith for said protein.

9. An antibody as claimed in Claim 8 which is a monoclonal antibody.

10. An antibody as claimed in Claim 8 or 9 which is a class M immunoglobulin with a kappa-light chain.

11. A fragment of the antibody of any of Claims 8 to 11, which fragment binds specifically to the protein of the invention.

12. A method of producing an antibody to a protein comprising:

- innoculating an animal with a protein according to any of Claims 4 to 7, wherein the protein elicits an immune response in the animal to produce the antibody; and

- isolating the antibody from the animal.

13. A method of producing an antibody as claimed in Claim 11 in which the animal is innoculated with G-CCM cells of ECACC deposit No. 86022702.

14. A method for producing a hybridoma, comprising the step of innoculating a suitable subject with a protein according to any of Claims 4 to 7, or an antigenic fragment thereof, and fusing cells from the subject with a myeloma cell to produce the hybridoma.

15. A method according to Claim 14 in which the subject is innoculated with G-CCM cells of ECACC deposit No. 86022702.

16. A hybridoma cell obtainable according to the method of Claims 14 or 15.

17. A hybridoma cell of, or derived from, ECACC Deposit No. 03073001.

18. A monoclonal antibody obtainable from a hybridoma cell of, or derived from, ECACC Deposit No. 03073001.

19. A method of detecting an astrocytoma cell in a sample of human cells, which method comprises the step of contacting the cell sample with an antibody according to any of Claims 8 to 10, or 18, or a fragment thereof, and detecting those cells which have bound the antibody or fragment, wherein binding of the antibody or the fragment to a cell is indicative of an astrocytoma cell.

20. A method as claimed in Claim 19 in which the antibody is a monoclonal antibody.

21. A method of detecting a primary breast carcinoma cell in a sample of human cells, which method comprises the step of contacting the cell sample with an antibody according to any of Claims 8 to 10, or 18, or a fragment thereof, and detecting those cells which have bound the antibody or fragment, wherein binding of the antibody or the fragment to a cell is indicative of a primary breast carcinoma cell.

22. A method according to Claim 21 in which the antibody is a monoclonal antibody.

23. A diagnostic kit for diagnosing the presence of a cell selected from the group consisting of: astrocytoma cells; malignant melanoma secondary tumour cells; and primary breast carcinoma cells, the kit comprising a (primary) antibody according to any of Claims 8 to 10, or 18, or a fragment thereof.

24. A diagnostic kit as claimed in Claim 23 in which the antibody comprises a detectable label.

25. A diagnostic kit as claimed in Claim 23 in which the kit comprises a secondary antibody which specifically binds the (primary) antibody, which secondary antibody comprises a detectable label.

26. A biological targeting device comprising an antibody according to any of Claim 8 to 10, or 18, or a fragment thereof, and a therapeutic ligand.

27. A therapeutic antibody comprising an antibody according to any of Claims 8 to 10, or 18, or a fragment thereof.

28. A method of treating cancer in an individual by inducing apoptosis in cells in the individual which express an MQ1 protein, which method comprises a step of treating an individual with an antibody of any of Claims 8 to 10, or 18, or a fragment thereof.

29. A method according to Claim 28 in which the cancer is selected from the group consisting of: malignant astrocytomas ; malignant melanoma secondary tumours; and primary breast carcinomas.

30. A method according to Claim 28 or 29 in which the antibody is a monoclonal antibody.

31. A method as claimed in any of Claims 28 to 30 in which the antibody is humanised.

32. A polynucleotide which is anti-sense to an isolated nucleic acid sequence of any of Claims 1 to 3.

33. An anti-sense polynucleotide as claimed in Claim 32 comprising the sequence of Sequence ID No. 4.

34. An anti-sense polynucleotide as claimed in Claim 32 consisting of the sequence of Sequence ID

No. 4.

35. A method of treating cancer in an individual by inducing apoptosis in cells in the individual which express an MQ1 protein, which method comprises a step of treating an individual with an anti-sense polynucleotide of any of Claims 32 to 34.

36. A method according to Claim 35 in which the cancer is selected from the group consisting of: malignant astrocytomas; malignant melanoma secondary tumours; and primary breast carcinomas.

ABSTRACT

Cancer Diagnosis and Therapy

The invention relates to an oncofetal glycoprotein, referred to as MQ-1, nucleic acid sequences coding for the protein, and antibodies which bind specifically to the protein. Also described is a hybridoma capable of producing monoclonal antibodies which bind specifically to the protein of the invention. Methods, and kits, for diagnosing and treating cancer using the antibodies of the invention are also described. Anti-sense polynucleotides are also described, as are methods for inducing apoptosis in cells which express MQ-1.

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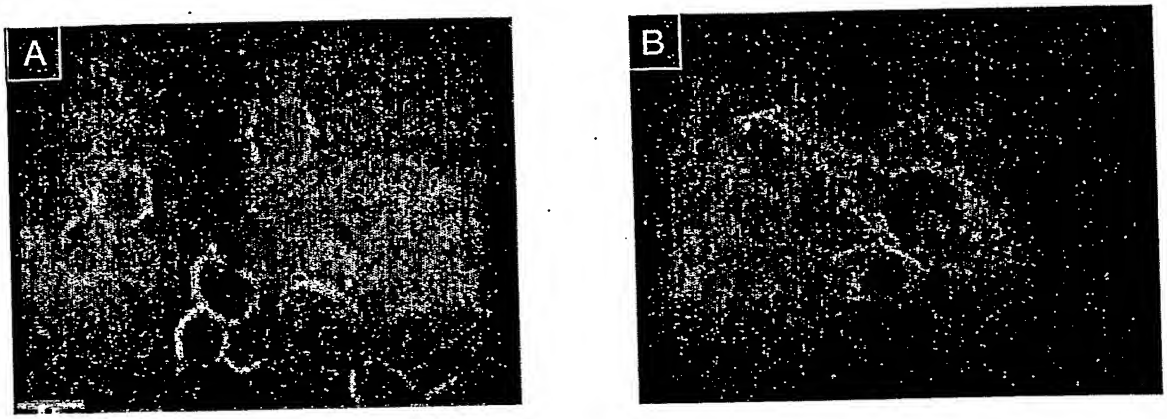
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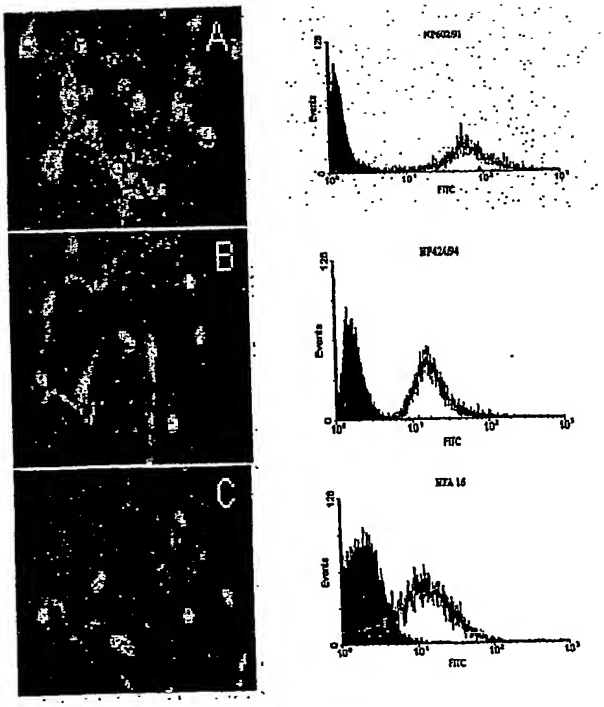
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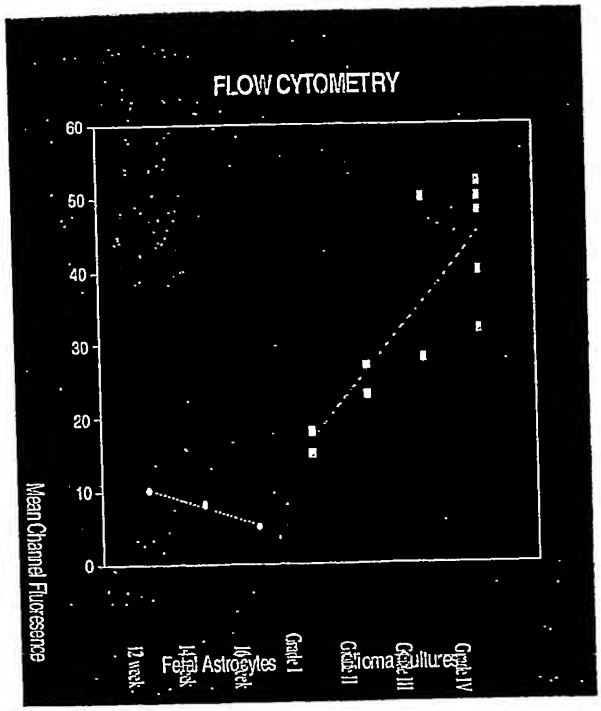
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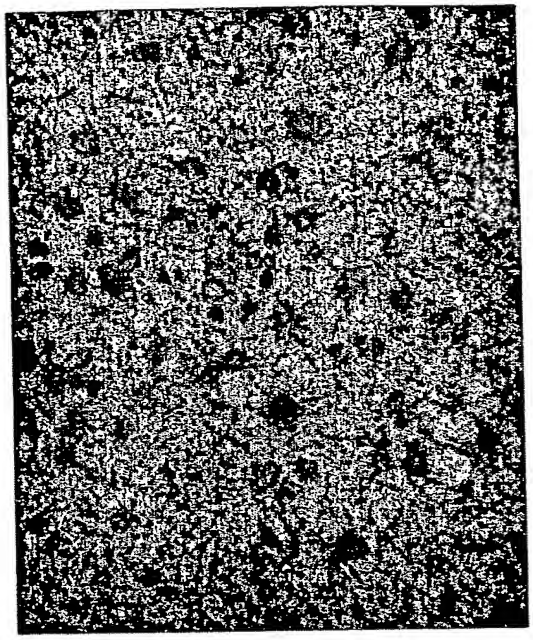

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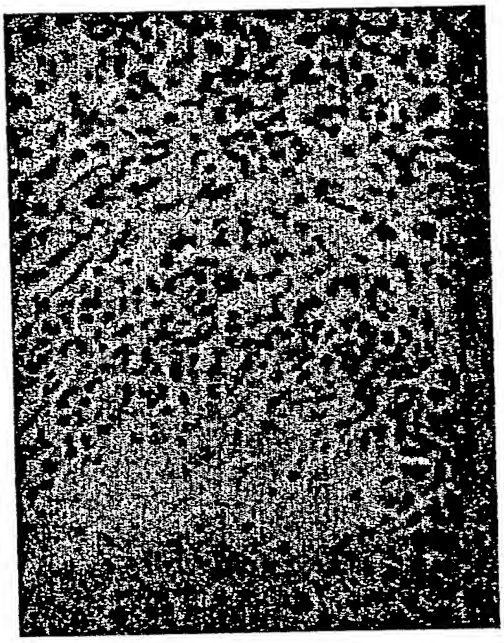

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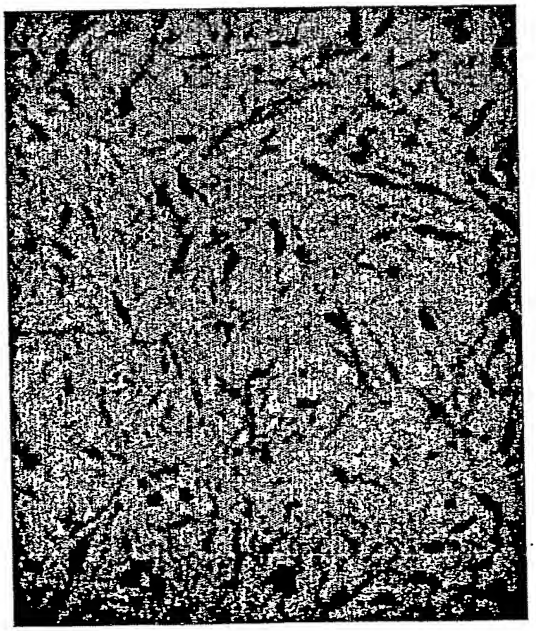

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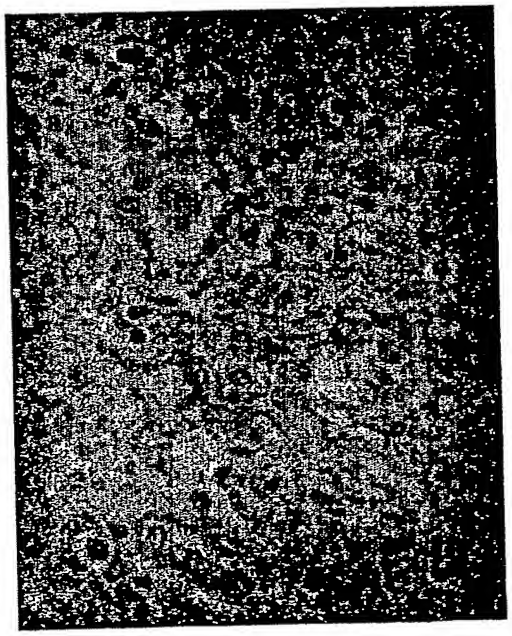
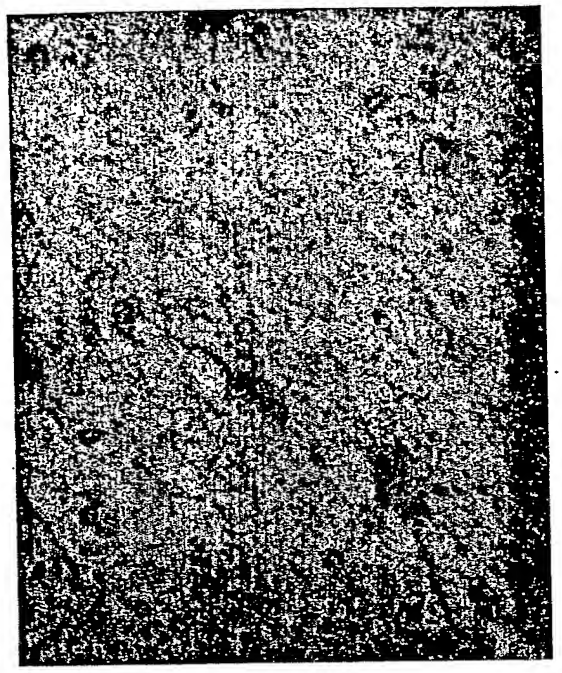


C

Fig. 3



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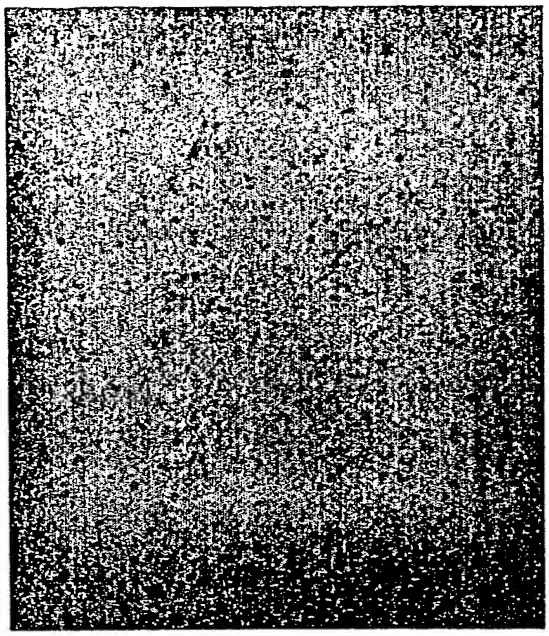
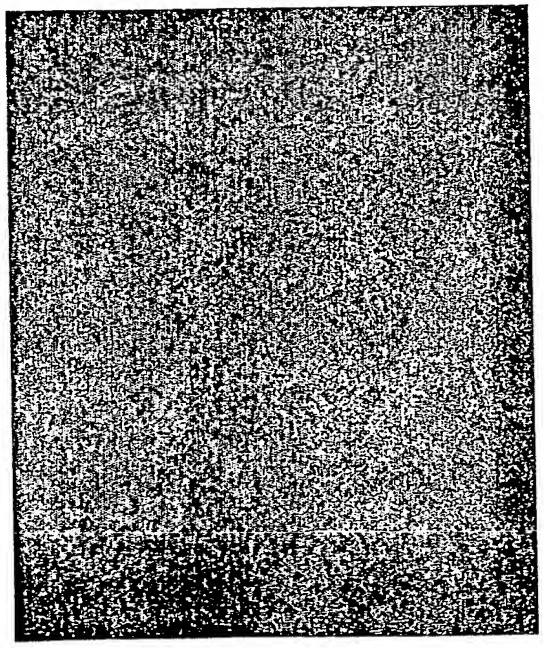
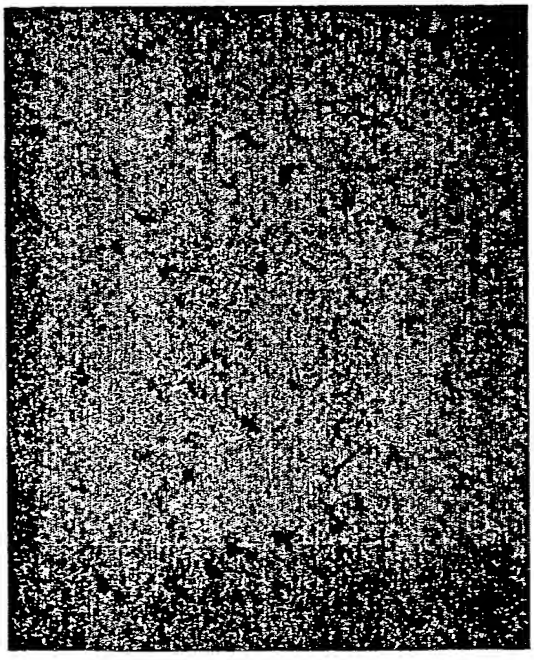


D



A

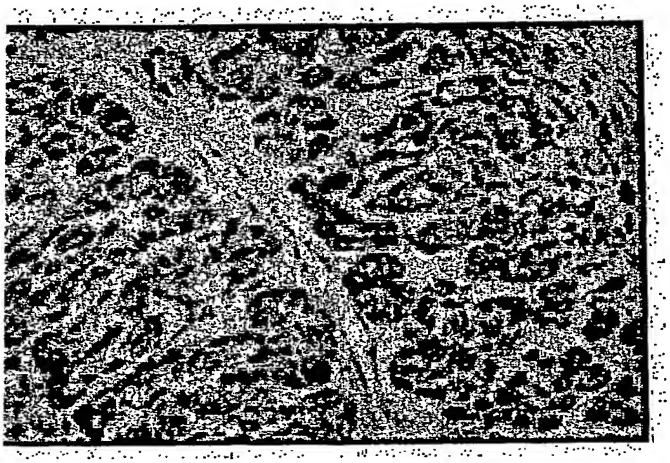


C

Fig. 4

Fig.5

A



B

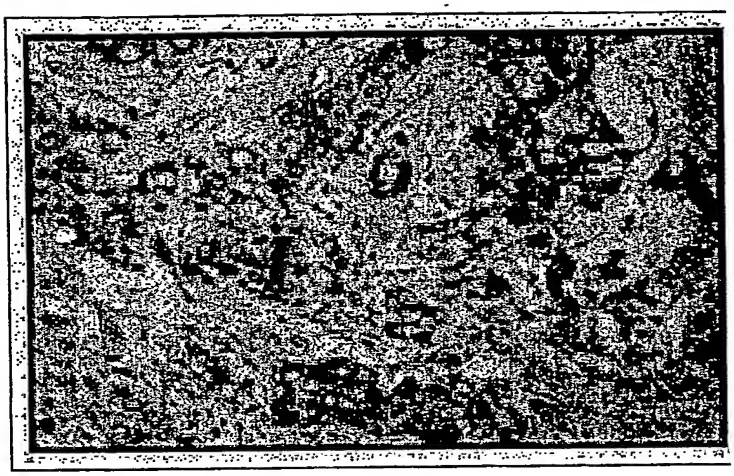


Fig.6

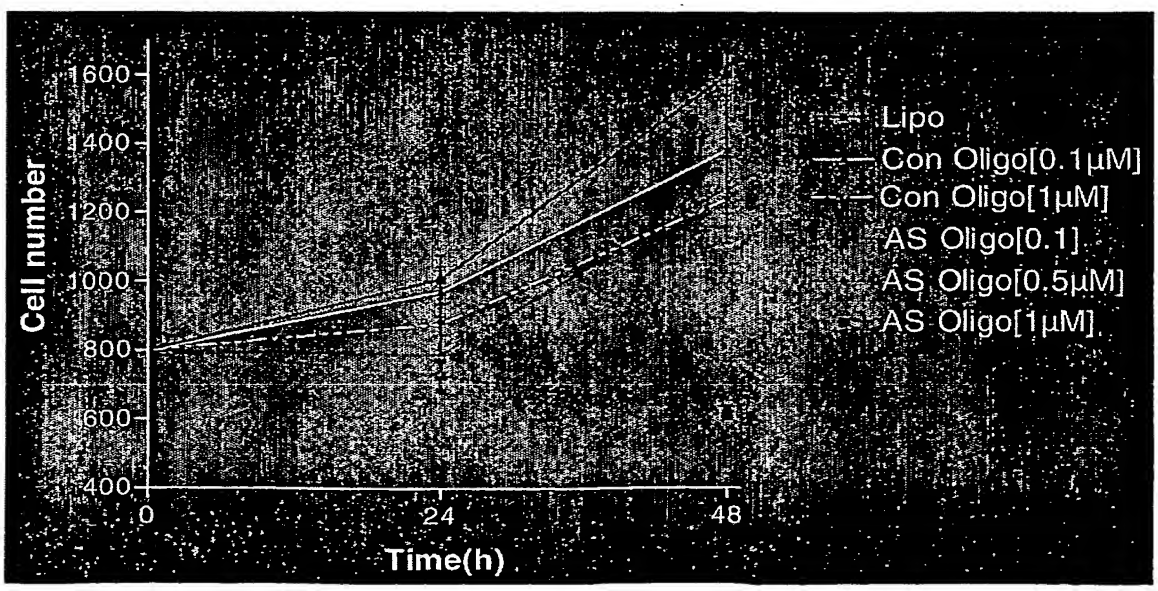

Cell number
1600
1400
1200
1000
800
600
400
0
24
48
Time(h)
Lipo
Con Oligo[0.1μM]
Con Oligo[1μM]
AS Oligo[0.1]
AS Oligo[0.5μM]
AS Oligo[1μM]

Fig. 7

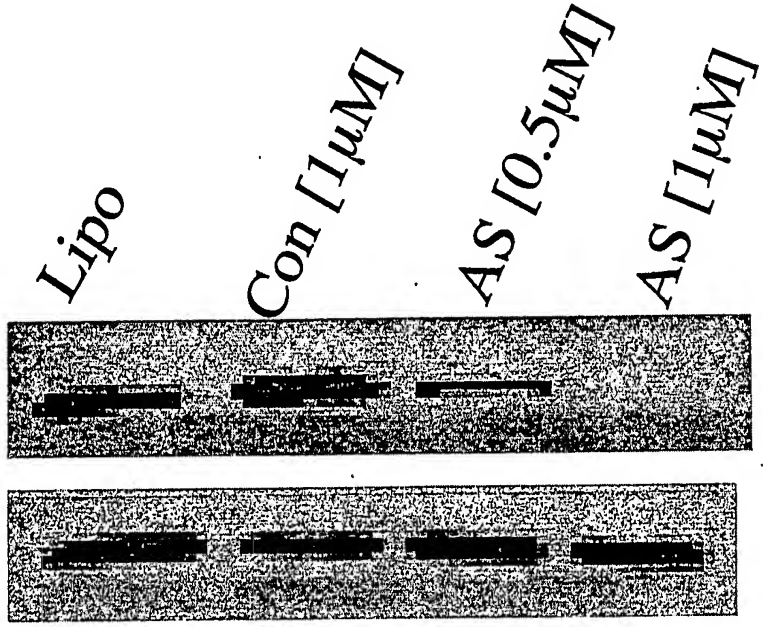

Lipo
Con [1μM]
AS [0.5μM]
AS [1μM]
PARP
B-TUBULIN

Fig. 8

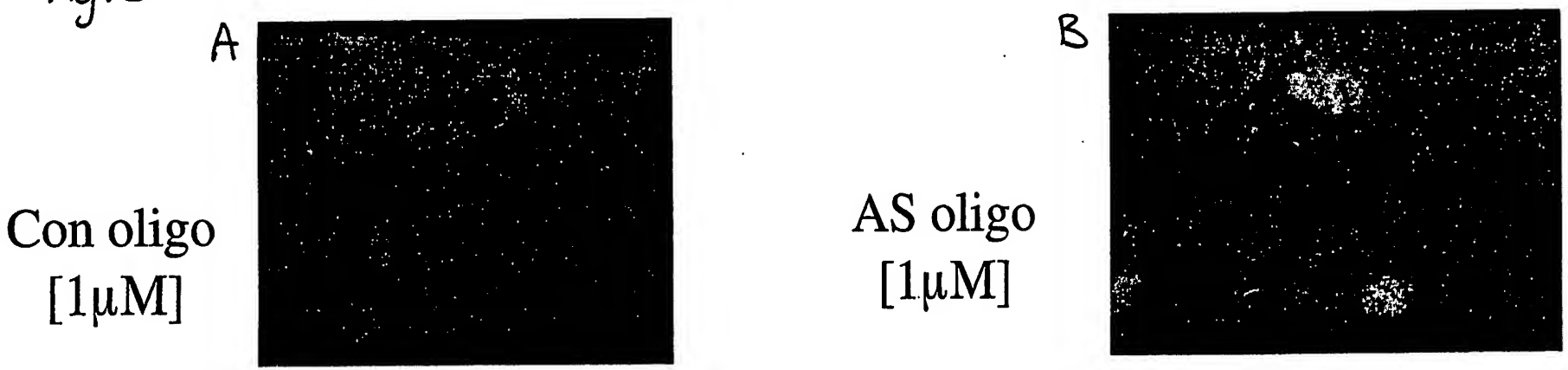
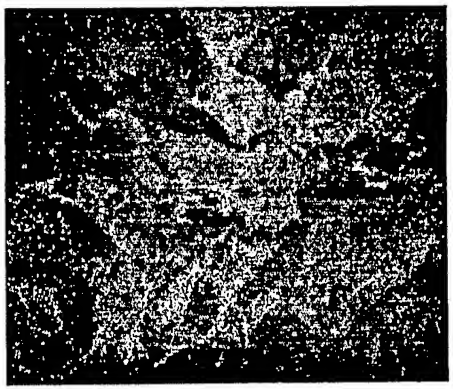

Cleaved Caspase 3 ICC
A
Con oligo
[1μM]
B
AS oligo
[1μM]

FIG. 9

A

Con Oligo [0.1μM]

B



Con Oligo [0.5μM]

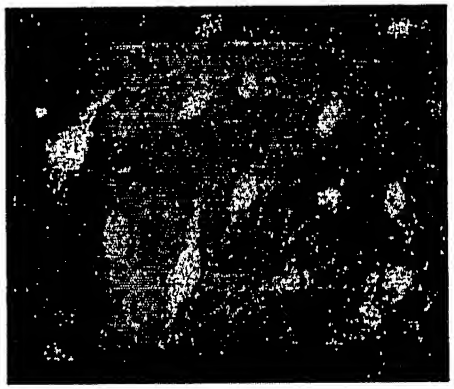
C

Con Oligo [1μM]

D

AS Oligo [0.1μM]

E



AS Oligo [0.5μM]

F

AS Oligo [1μM]